Environmental Microbiology (2004) 6(1), 1–11 doi:10.1046/j.1462-2920.2003.00539.x

Minireview

Are viruses driving microbial diversification and diversity?

Markus G. Weinbauer* and
Fereidoun Rassoulzadegan
Laboratoire d'Océanographie de Villefranche (LOV), Biogeochemistry, Functional Diversity and Microbial Ecology Group, BP 28, 06234 Villefranche-sur-Mer, France.

Summary

Viruses can influence the genetic diversity of prokaryotes in various ways. They can affect the community composition of prokaryotes by 'killing the winner' and keeping in check competitive dominants. This may sustain species richness and the amount of information encoded in genomes. Viruses can also transfer (viral and host) genes between species. Such mechanisms have probably influenced the speciation of prokaryotes. Whole-genome sequencing has clearly revealed the importance of (virus-mediated) gene transfer. However, its significance for the ecological performance of aquatic microbial communities is only poorly studied, although the few available reports indicate a large potential. Here, we present data supporting the hypothesis that viral genes and viral activity generate genetic variability of prokaryotes and are a driving force for ecological functioning and evolutionary change.

Introduction

Viruses are the most abundant 'life forms' in aquatic systems, and their number probably exceeds 10^{29} in the ocean (Wilhelm and Suttle, 1999). Aligning these oceanic viruses and assuming an average diameter of 50 nm for a marine virus, the viral string-of-pearls would be 400 000 light years long. In comparison, the diameter of our galaxy, the Milky Way, is only 25 000 light years. The huge number of viruses found in aquatic systems has prompted research showing that they play a significant role in the transfer of matter and energy in aquatic microbial food webs. For example, viral lysis is a major cause of prokaryotic mortality in aquatic systems, and equals on average grazing-induced mortality (Fuhrman, 1999; Wommack and Colwell, 2000). The fate of bacterioplankton production influences the rate of food web processes, as grazed production enters 'higher' trophic levels (grazing food chain), whereas lysed production is available to other bacterial cells. At the community level, viruses might even promote prokaryotic production and respiration (Fuhrman, 1999) and thus bacterial ecosystem functions such as organic matter oxidation. Viruses also infect unicellular planktonic organisms such as flagellates and algae. In general, viral lysis affects the carbon and nutrient flow through different compartments of the food web. In marine pelagic systems, between 6% and 26% of the photosynthetically fixed carbon is channelled or 'shunted' to the dissolved organic matter (DOM) pool by viral lysis of cells at all trophic levels (Wilhelm and Suttle, 1999). The carbon stored in the DOM pool equals that in atmospheric CO_2 (Hedges, 1992), suggesting that viral infection of marine prokaryotes and phytoplankton has an influence on global carbon cycling and climate. Viruses may not only influence the flow of energy and matter through food webs, but also play a significant role in the transfer of information encoded in DNA.

Some viral groups such as the Caudovirales, the tailed double-stranded (ds)DNA phages, are probably older than the separation of life into the three now recognized domains of life (Ackermann, 1999; Hendrix, 1999). For example, the same three families of tailed phages infect cyanobacteria and heterotrophic bacteria suggesting that these viruses existed before cyanobacteria split from the rest of the prokaryotes (Suttle, 2000a). Before the occurrence of eukaryotic grazers such as flagellates and ciliates, viruses were probably the main predators of cells in the prokaryotic world. The early appearance of viruses in the evolution of life has resulted in sophisticated interactions between viruses and their prokaryotic hosts. Such interactions range from true parasitism such as in chronic infection, where viruses are released from cells without

Received 5 May, 2003; accepted 22 September, 2003. *For correspondence. E-mail Markus.Weinbauer@obs-vlfr.fr; Tel. (+33) 4 93 76 38 55; Fax (+33) 4 93 76 38 34.

© 2004 Blackwell Publishing Ltd

killing hosts, to predation such as in lytic infection of unicellular organisms resulting in cell death. A mutualistic interaction might occur in some forms of lysogeny, where viral genomes stay within the host genome and confer new metabolic traits to hosts (phage conversion), which can increase the fitness of the host and thus also the survival rate of the phage. A vast variety of interactions with viruses is also known for uni- and multicellular eukaryotes. How these interactions determine the way viruses influence prokaryotic diversity and gene flux in aquatic systems is the topic of this review. A summary of such influences is presented in Fig. 1.

Studies on prokaryotic diversity have been implemented in research on microbial ecology and biogeochemistry predominantly as a result of the groundbreaking work of Carl Woese, who has shown using sequence data from the small subunit (SSU) of the ribosomal RNA operon that life consists of three domains, Bacteria, Archaea and Eukarya (Woese, 1987; Wheelis *et al*., 1992). Molecular approaches have been used to circumvent the great plate count anomaly meaning that typically <1% of aquatic bacteria can be cultivated on culture plates (Staley and Konopka, 1985), and numerous as yet uncultured species have been detected (Giovannoni and Rappé, 2000). These studies have also renewed interest in culturing the uncultured species, and considerable progress has been made in this field. For example, 16S rRNA gene analysis has revealed the presence of a ubiquitous and abundant as yet uncultured bacterial group of Alphaproteobacteria, the SAR11 cluster (Morris *et al*., 2002). Recently, refined culturing techniques have allowed the isolation of a strain from this cluster (Rappé *et al*., 2002). Also, the finding of a significant contribution of *Actinobacteria* to total bacterial abundance (up to 60%) in freshwaters using fluorescent *in situ* hybridization (FISH) (Glöckner *et al*., 2000) has provoked interest in this group and resulted in the isolation of the first freshwater members of the class *Actinobacteria* (Hahn *et al*., 2003).

A genuine feature of prokaryotes is their ability to exchange genes. Horizontal or lateral gene transfer (LGT) or 'microbial sex' results from conjugation (gene transfer directly between two cells), transduction (phage-mediated gene transfer) and transformation (gene transfer by uptake of free DNA such as plasmids) (Paul, 1999). Also, there is gene flux along cell lines due to multiplication of genomes and cell division (vertical gene transfer, VTG).

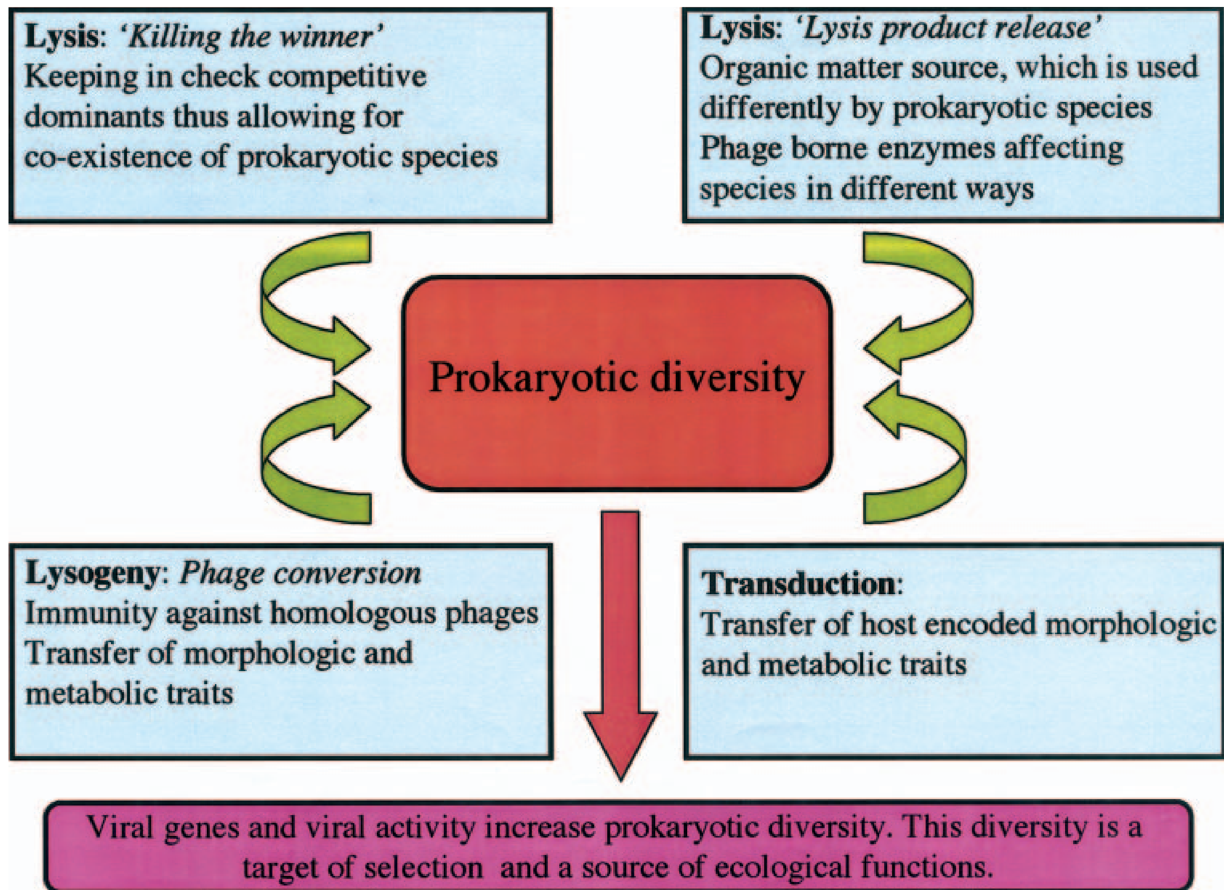

Fig. 1. Summary of potential effects of viruses on prokaryotic diversity.

Microbial diversity

Diversity can be described in two ways, as species diversity or biodiversity. Species diversity consists of three parameters, species richness (the number of species), species evenness (the significance of species in terms of abundance, biomass or activity) and species difference (the taxonomic relatedness). These parameters can be used to calculate diversity indices; however, they are not routinely applied to microbes. Biodiversity might be described as the total (structural and functional) diversity of life (Wilson, 1992). In other terms, diversity is the amount and distribution of (genetically encoded) information or the 'dictionary of living nature' (Margalef, 1997). Microbial biodiversity is even less known than species diversity; however, metagenomics (community genome sequence analysis) provides us with a tool to start tackling this Herculean task.

Prokaryotic diversity

Prokaryotic classification and the prokaryotic species concept are controversial topics. This is partially because of technological progress, which results in a reconsideration of such concepts (Rossello-Mora and Amann, 2001), but it has also been argued that a more 'natural view' of microorganisms should be adopted regarding species in their ecological and evolutionary context (Ward, 1998). DNA–DNA hybridization is a crucial parameter for a species definition of prokaryotic isolates; however, it is not sufficient alone to define a 'species' in a strict taxonomic sense. A 70% DNA–DNA hybridization, which corresponds to ≈ 96% sequence similarity, is typically used as delineation between species (Stackebrandt and Goebel, 1994). 16S ribosomal RNA gene analysis is the most widely used method of estimating diversity in environmental samples. This is based on the observation that a threshold of 97% similarity of the entire 16S rRNA gene often matches the concept of 70% DNA–DNA hybridization (Stackebrandt and Goebel, 1994). However, growing evidence suggests that this threshold might be higher (Rossello-Mora and Amann, 2001). Assessing species richness based on 16S rRNA gene sequences is sometimes regarded as a futile enterprise because of selective amplification of sequences and the presence of multiple operons, which can differ with respect to their sequence (Wintzingerode *et al*., 1997; Dahllof *et al*., 2000). Also, rare species are probably not detected by the methods used. Moreover, it has been argued that sequence differences might not always correspond to different species and that the phylogeny of a gene, such as encoding for the 16S rRNA gene, might not match the phylogeny of the genome or cell line of a species. For example, data on lateral gene transfer, such as mediated by viruses, have even invoked questions on the validity of the universal tree of life. LGT may 'shake the tree of life' (Pennisi, 1998), as swapping of genes among species could make a universal classification based on phylogenetic reconstructions impossible (Doolittle, 1999; Martin, 1999). Finally, 16 rRNA gene sequences alone are not a valid tool for bacterial species description *sensu strictu* (Rossello-Mora and Amann, 2001). However, 16S rRNA gene approaches may serve as a pragmatic way of determining the number of phylotypes in the environment. Indeed, it has been argued that the methods that microbial ecologists are applying to assess species diversity are often roughly as precise as methods used by ecologists of eukaryotes (Hughes *et al*., 2001). Thus, the phylogeny of a conserved gene (or several conserved genes) may be used for reconstructing phylogenetic relatedness, and the phylogeny of non-conserved genes may reflect evolutionary plasticity and adaptation. Analysing both types of genes then becomes useful in tackling species and biodiversity of prokaryotes.

Culture-independent 16S rRNA gene and other DNA-based method have provided us with an estimation of the number of 'types' of prokaryotes in various environments, which will be used as an indication of prokaryotic species richness in this review. Using various ways to estimate richness, between a few and 160 bacterial species can be expected in a bulk water sample of several to tens of litres from marine or freshwater pelagic systems (Table 1). This concurs well with a maximum possible richness of 163 species per water sample estimated on the basis of log-normal species abundance curves (Curtis *et al*., 2002).

Attempts have been made to estimate prokaryotic species richness in parts of the ocean or in the entire ocean.

Table 1. Estimation of bacterial species richness in aquatic systems.

No. of species	Method	Remarks	Reference
Up to 31	DGGE	Coastal ecosystem	Murray *et al.* (1996)
54–95	Clone libraries	Low to high productivity ponds	Hughes *et al.* (2001)
About 100	Prediction on viral infection	Mathematical model	Thingstad and Lignell (1997b)
160	DNA reassociation kinetics	Estuarine waters	Torsvik *et al.* (2002),
163	Log-normal abundance species curves	Theoretical maximum	Curtis *et al.* (2002)

Studies were selected (i) to represent different approaches and (ii) to show high species richness, as many approaches probably underestimate 'true' species richness.
DGGE, denaturing gradient gel electrophoresis.

© 2004 Blackwell Publishing Ltd, *Environmental Microbiology*, **6**, 1–11

Using entire 16S rRNA gene data from cultured and uncultured sequences found in GenBank and a species threshold of 97% sequence similarity, it was suggested that there are about 1200 species in the surface ocean (Hagström *et al.*, 2002). If this number holds roughly true, we have not detected the tip but the bulk of the iceberg of species richness in marine surface waters. However, using log-normal species abundance curves, the maximum possible species richness in the entire ocean should be $< 2 \times 10^6$ (Curtis *et al.*, 2002). Archaea, which are as abundant as Bacteria in the deep ocean and may account for about one-third of the pelagic prokaryotes in the world's oceans (Karner *et al.*, 2001), cannot be responsible for this difference, as their maximum richness was estimated to be only 20 000 species (Curtis *et al.*, 2002). Although such data provide first insights into prokaryotic diversity, assessing the 'true' species richness in single samples and in ecosystems remains a challenge for future research.

Genome complexity of prokaryotes in pelagic systems is basically unknown. Torsvik *et al.* (2002) have estimated the genome complexity of communities in marine sediments based on genome equivalents relative to the *Escherichia coli* genome and assessing bacterial species by reassociation kinetics of DNA. Genome complexity was 4.8×10^{10} bp in a pristine sediment and 2×10^8 bp in marine fish farm sediment. The genome complexity of microbes in the fish farm sediment is probably similar to pelagic systems, as about 50 genome equivalents were present, which falls within the range of species richness of marine bacterioplankton (Table 1). Assuming that there are a maximum of 163 'species' or genome equivalents in a sample (Curtis *et al.*, 2002) and using the genome size of *E. coli*, maximum genome complexity would be 6.7×10^8 bp in a given water sample. However, as the genome of an average marine bacterium might be smaller than that of *E. coli* (Button *et al.*, 1998; Rappé *et al.*, 2002), this might be a slight overestimation. Using the estimates of species richness outlined above, the genome complexity in the surface ocean would be at least 4.9×10^9 bp, and genome complexity in the entire ocean should be $< 8.2 \times 10^{12}$ bp. These estimates are uncertain because of the unknown number of species and the unknown genetic variability within and between species. Although genetic variability within species can be large, it should be smaller than among species. If not, concepts such as DNA similarity, e.g. a 70% DNA–DNA hybridization, would make no sense as crucial parameters for species descriptions. Despite these uncertainties, such estimates may describe the possible range of prokaryotic genome complexity in the ocean.

Viral diversity and diversification

The species concept has been applied to viruses by the International Committee on the Taxonomy of Viruses (ICTV) (Regenmortel, 1992). Recently, Rohwer and Edwards (2002) have proposed a genome-based taxonomy for phages based on the overall sequence similarity of predicted protein sequences of 105 completely sequenced phages. This taxonomy is roughly similar to the one proposed by the ICTV, although there are also some differences. It has also been argued that a definition of viral species is not meaningful in the presence of excessive gene transfer (Lawrence *et al.*, 2002).

Assessing viral diversity in the environment is difficult. This is because of not only conceptual difficulties in defining a viral species but also the great plate count anomaly, as hosts have to be cultured before phages can be isolated. Moreover, there is no common molecule for viruses, not even for the tailed dsDNA phages, which could be used in an analogous way to the 16S rRNA gene for cellular microorganisms (Hendrix *et al.*, 1999). However, it might be possible to use conserved or 'core' genes as genetic markers for different viral groups. Sequence differences of such genes would then correspond to genotypes, and the number of genotypes can be used as an estimation of viral species richness. Genetic marker molecules have been found in several viral groups. For example, primers are available for algal viruses (Phycodnaviridae), targeted against the DNA polymerase gene (Chen and Suttle, 1995; Short and Suttle, 2002), and for cyanophages, targeted against the gene of the g20 capsid protein (Fuller *et al.*, 1998; Wilson *et al.*, 1999; Zhong *et al.*, 2002), although there is some evidence that the cyanophage primers also cover some other phages. Techniques such as denaturing gradient gel electrophoresis (DGGE) or clone libraries were used to assess genotype richness of cyanophages and algal viruses (Short and Suttle, 2002; Zhong *et al.*, 2002). Also, a community approach has been developed based on the separation by size of viral genomes using pulsed-field gel electrophoresis (PFGE; Wommack *et al.*, 1999a,b). The number of viral genotypes, estimated by the number of genome size classes, is conservative because more than one genotype might be hidden in a single band.

Assuming for the sake of argument that every prokaryotic species typically has at least one specific virus, we can make a rough estimation of viral diversity. Using this line of argument, species richness of viruses should be at least as high as the species diversity of microbes. Using various approaches, many of them only targeting algal or cyanophage diversity, up to 36 different viral types were found in water samples (litres to tens of litres) from marine systems (Table 2). Recently, assessing the metagenome of two natural viral communities from 200 l of coastal waters using shotgun cloning, Breitbart *et al.* (2002) have estimated between 374 and 7114 viral genotypes in the samples. This number is higher than that for bacterial

Table 2. Estimation of viral genotype richness in marine systems.

No. of types	Method	Remarks	Reference
33	DGGE	Algal viruses; all sequenced bands from all environments	Short and Suttle, (2002)
13–29	Clone library	Cyanophages; 114 different clones	Zhong *et al.* (2002)
7–36	PFGE	Various environments	Steward and Azam (2000); Steward *et al.* (2000)
374–7114	Metagenomics	Shotgun cloning of two marine samples	Breitbart *et al.* (2002)

Note that two approaches were targeted to specific viral groups, the algal viruses and cyanophages.
DGGE, denaturing gradient gel electrophoresis.
PFGE, pulsed-field gel electrophoresis.

species, thus supporting the idea that viral diversity is enormous and higher than that of cellular microbes. Curiously, the diversity of phages, as assessed by the metagenomics approach, is about an order of magnitude higher than the diversity of microbes (Tables 1 and 2), concurring with a viruses-to-bacteria ratio of 10 typically found in coastal surface waters (Wommack and Colwell, 2000). This would indicate that there are on average about 10 specific viruses per bacterial species, which corresponds well with data from isolated bacteriophages (Weinbauer, 2003). At a viral abundance of 10^7 viruses ml^{-1} for coastal systems, the average abundance of single viral genotypes would be $\approx 10^4$ ml^{-1}. Using an average genome size of 5×10^4 bp for a marine virus, as determined by PFGE of viral communities (Steward and Azam, 2000) and the species richness data from the metagenomics approach, a genome complexity for viruses between 1.5×10^7 bp and 3.5×10^8 bp per sample can be calculated. These numbers are close to the maximum estimated genome complexity of bacterioplankton per sample. Thus, there seems to be a comparable genome complexity of viruses and prokaryotes in a system.

Viruses are a source of diversity and harbour specific genes. These particular genes involve structural genes (e.g. encoding for capsid and tail fibre proteins), genes encoding for insertion sites into the host genome or enzymes to lyse host cells. Other unique genes enable a lytic phage to infect a lysogenized host. Viruses can also carry genes for repairing DNA damage. For example, homologues of the *DenV* gene originally found in the T4 phage were also detected in *Chlorella* viruses, i.e. viruses infecting a symbiotic alga (Furuta *et al.*, 1997). These genes have no resemblance to genes of cellular organisms. Whole-genome sequencing of marine viral isolates and metagenomics of viral communities also suggest a predominance of genes specific for viruses (Rohwer *et al.*, 2000; Breitbart *et al.*, 2002; Chen and Lu, 2002; Paul *et al.*, 2002). In general, databank analysis of genes has shown that phage and viral genes were not stolen from hosts, but '. . . the large majority of phage and viral genes are unique to various families of viruses, not hosts . . .' (Villarreal, 2001).

The genomes of the Caudovirales seem to be composed of modules such as head assembly, tail assembly or lysogeny and lysis cassettes, which consist of genes belonging together functionally (Hendrix *et al.*, 1999; 2000; Lawrence *et al.*, 2002). This mosaic organization of the viral genome favours the exchange of functional genetic units between phages. Such events will occur most frequently during a co-infection with two or more phages or when a phage infects a cell containing a prophage (Moineau *et al.*, 1994; 1995). Thus, cells might be 'phage factories', which release a wide variety of recombinant phages in the environment (Ohnishi *et al.*, 2001), and these chimeric phages might finally result in new viral species.

Vertical gene flux

The genome of a species exists in multiple copies depending on the abundance of cells of this species, and VGT rates are determined by the doubling times. There is also a genomic variability of populations within a species; however, this is not considered further here, as the amount of this variability is not known for major bacterial types in aquatic systems. Data on the standing stock and genome transfer rate of species have not been estimated in aquatic systems because of methodological problems of determining cell abundance, genome size and doubling times of species *in situ*. For natural communities, only a few studies have estimated the doubling rates and abundance of cells at roughly the species level (Weinbauer and Höfle, 1998; Pinhassi *et al.*, 1999). Knowing the genome size, the transfer rate of DNA-encoded information can be estimated. Owing to the development of molecular tools applicable to natural communities such as FISH and quantitative polymerase chain reaction (PCR), the partitioning of standing stocks and fluxes of DNA into genome equivalents (species) and its relation to genome complexity seems to be a task that can be tackled now or in the near future. Viruses can influence these fluxes. For example, viral lysis can reduce the vertical information transfer of prokaryotic genomes by removing those cells of a species susceptible to infection.

Vertical gene flux in viruses depends on their life cycles. In chronic infection, genomes are produced by releasing viruses from cells without killing them. Lytic phages release progeny genomes in bursts as the cell lyses and, thus, VGT is the cell lysis rate times the burst size times the genome size. For prophages of lysogenic prokaryotes, the viral genome replicates along with the host genome and, thus, VGT is the doubling rate of the host times the genome size of the provirus. We are not aware of such data for single viral genotypes in aquatic systems.

Viral impact on prokaryotic diversity

The finding of high viral numbers and high viral infection frequencies of bacteria in aquatic systems (Bergh *et al*., 1989; Proctor and Fuhrman, 1990; Suttle *et al*., 1990) has prompted the idea that they might influence species diversity (Fuhrman and Suttle, 1993; Thingstad *et al*., 1993). This has been elaborated in the concept of 'killing the winner' (Thingstad and Lignell, 1997a), meaning that lytic viruses can keep in check competitive dominants and thus might allow for the co-existence of less competitive populations and even sustain bacterial diversity. This model is supported by the finding from isolated phage–host systems that phages propagate as a function of host density and can thus control host abundance. For example, Hennes *et al*. (1995) added the *Vibrio natriegens* strain PWH3a to sea water from which it was previously isolated. After a bloom of the strain, indigenous phages could terminate the bloom, resulting in a strong increase in the abundance of phages being able to produce plaques on culture plates with this strain. In experiments with several phage–host systems, phages changed the clonal composition of the hosts by forcing the formation of resistance mechanisms (Middelboe *et al*., 2001). Virus-induced resistance has also been reported for cyanobacteria in laboratory and *in situ* studies (Waterbury and Valois, 1993; Suttle and Chan, 1994). Other evidence comes from the geographical and temporal variability of viral populations in Chesapeake Bay (Wommack *et al*., 1999a,b) and from the dynamics of viral populations in mesocosms (Larsen *et al*., 2001; Øvreås *et al*., 2003) as determined by PFGE. These data are compatible with predator–prey-type oscillations of different phages and their hosts, whereas total abundance of bacteria and viruses remains relatively stable (Wommack *et al*., 1999a,b). Incubating a freshwater bacterial community with and without the viral community from the same sample resulted in changes in abundance and growth rates of bacterial populations as well as in changes of the community composition (Weinbauer and Höfle, 1998). In similar experiments, phytoplankton (Suttle, 1992) or plankton community composition (Peduzzi and Weinbauer, 1993a) was influenced by adding viruses. Such data support the 'killing the winner' concept, although we still lack detailed *in situ* information such as on the dynamics of phage–host systems within communities.

A virus-induced mass lysis event of filamentous cyanobacteria caused great changes in community composition (Van Hannen *et al*., 1999). Although the 'killing the winner' concept might also hold true in this case, it is also possible that the mass release of organic matter during cell lysis changed the composition and bioavailability of organic nutrients and thus caused changes in the community composition of bacteria. It has been shown that changes in the DOM composition can indeed affect bacterial community structure (Lebaron *et al*., 1999; Riemann *et al*., 2000; Arrieta and Herndl, 2002). An influence of viruses on bacterial community composition by release of organic matter has been also suggested by Middelboe (2000). Other lysis products such as phage-borne enzymes can kill cells (Fuhrman and Noble, 2000), which might also influence community composition. These 'lysis product mechanisms' could enhance or mask the 'killing the winner' effect depending which species they act upon.

Some phages do not cause immediate lysis, but replicate along with the host cell, e.g. by integrating their genome into the host genome. Such lysogenized cells are immune against infection by homologous phages and, sometimes, the phages also confer morphological, metabolic or immunogenic properties to the host. A spectacular example of phage conversion are *Vibrio cholerae* strains, which only cause cholera when they are infected with a lysogenic phage carrying the cholera-causing toxin (Waldor and Mekalanos, 1996). Phage conversion can increase the fitness of cells (Edlin *et al*., 1975; Lin *et al*., 1977), which could influence community composition by allowing for the survival or dominance of such converted cells. The percentage of lysogenized cells in bacterioplankton varies greatly in surface waters (Jiang and Paul, 1996; Weinbauer and Suttle, 1996); however, it has been shown recently that the majority of bacterioplankton from deep waters in the Mediterranean Sea are lysogenized (Weinbauer *et al*., 2003). Thus, immunization against infection and phage conversion are possible ways in which phages can influence diversity in this environment.

Virus-mediated gene transfer

One of the most surprising findings of whole-genome sequencing is the enormous extent of LGT, indicating that gene swapping among organisms is not a laboratory oddity but a constitutive factor in diversification. On average, 2.6 prophages have been detected per free-living bacterial species (Lawrence *et al*., 2002), and a number of bacterial genomes contain between 3% and 10% prophage DNA (Brüssow and Hendrix, 2002). An educated guess suggests that *E. coli* and *Salmonella* separated 100

million years ago. About 10% of the *E. coli* genome consists of genes that were acquired in more than 200 events of LGT after this divergence (Lawrence and Ochman, 1998). *E. coli* has an extremely variable genome size ranging from 4.5 to 5.5 Mb, although the rRNA operon is highly conserved. The genome of the *E. coli* strain O157 Sakai consists of a huge amount of strain-specific DNA (1.44 Mb) and contains 18 prophages or prophage remnants accounting for ≈50% of the strain-specific sequences. Moreover, six large chromosome segments, which seem to represent prophage-like genetic elements, are found in O157 (Hayashi *et al*., 2001). This indicates a high rate of virus-mediated LGT. Thus, phage genomes are an integral part of O157 genomes, suggesting that phages played a predominant role in the emergence of this strain (Ohnishi *et al*., 2001). As other strains also contain large numbers of prophages, prophage remnants and phage-related elements, bacteriophages appear to be major contributors to the genome diversification of *E. coli*. This is an example of how phages might have influenced evolutionary diversification and speciation. However, there are also ways in which viruses can influence the standing stock of information encoded in prokaryotic species at ecological scales.

Virus-mediated LGT has been documented in keystone studies from freshwater and marine systems (Saye *et al*., 1987; Jiang and Paul, 1998; Paul, 1999); however, transduction rates are difficult to measure *in situ*. In Table 3, transduction rates are listed, which were assessed using either natural bacterial or viral communities. The transduction frequency varied over orders of magnitude from 10^{-8} to 10^{-5} per virus. Jiang and Paul (1998) estimated that there might be up to 100 transductants l^{-1} day^{-1} or up to 1.3×10^{14} transductants per year in Tampa Bay. Based on estimates of the percentage of lysogenized cells in bacterioplankton along a depth profile down to 2000 m, estimates of bacterial production and burst size (Weinbauer *et al*., 2003), assuming low spontaneous induction frequencies of 10^{-5} per cell (Ackermann and DuBow, 1987), a conservative number of transductants per phage of 10^{-9} and the volume of the Mediterranean Sea (3.75×10^{6} km^3), we estimated 10^{13} transductants per year in the Mediterranean basin. These estimates suggest (in the absence of hard data) that transduction could be a significant and overlooked mechanism in microbial ecology. A similar line of argument has been put forward by Fuhrman (1999), suggesting that LGT is significant in the ocean. The shear abundance and diversity of viruses in the 'viriosphere' (C. Suttle, personal communication) makes virus-mediated LGT a potentially significant process.

The most surprising results, however, were reported for auxotrophic *E. coli* cells, to which amino acid prototrophy could be transferred by marine phages at the extremely high rates of up to 2.6×10^{-3} per virus (Chiura, 1997; Chiura *et al*., 2000). This transfer of DNA from marine phages to a non-marine enterobacterium could result from broad-host-range transduction. Although it is often assumed that phages do not trespass the genus barrier (Ackermann and DuBow, 1987), this concept has been questioned. Jensen *et al*. (1998) argued that narrow host ranges are an isolation artifact, and Wichels *et al*. (1998) have shown that host range can vary greatly between marine phages. A large variability of host ranges has also reported for cyanophages (Suttle, 2000b).

Microorganisms are not distributed homogeneously in aquatic systems, but they are concentrated in activity hotspots such as organic aggregates or around decaying phytoplankton cells (Azam, 1998; Long and Azam, 2001). In organic aggregates, not only bacteria but also viruses can be highly concentrated (Peduzzi and Weinbauer, 1993b; Simon *et al*., 2002), and infection frequencies of prokaryotes can be as high as in the surrounding water (Proctor and Fuhrman, 1991). Enhanced transduction rates were found on suspended material in freshwater probably because of a higher contact rate between bacteria and phages (Ripp and Miller, 1995). Thus, hot-spots of activity could also be hot-spots of transduction and lateral gene transfer in general. Protists acquire phages along with ingested bacteria (González and Suttle, 1993) and infected cells, and induction of lysogens in food vacuoles of a ciliate has been documented (Clarke, 1998). This could be a way of transferring phage DNA to eukaryotes. Indeed, protists might be what they eat (Doolittle as cited in Pennisi, 1998). Moreover, during viral lysis of cells, host DNA is released, which might stimulate DNA transfer by transformation.

The modular organization of biochemical pathways in the genome of prokaryotes is a prerequisite for successful gene transfer. Entire functions can be exchanged by a

Table 3. Transduction rates in marine systems.

Environment	Transducing frequency (no. of transductants/pfu or virus)	Transducing phage	Reference
Tampa Bay	1.57×10^{-8}	T-fD1B	Jiang and Paul (1998)
Gulf of Mexico (1500 m)	3.7×10^{-8}	UV T-fD1B	Jiang and Paul (1998)
Mediterranean Sea	3.0×10^{-5}–5.7×10^{-5}	Natural virus community	Chiura *et al*. (2000)

Only studies were considered in which either viral or bacterial communities were used.

single transfer event. Other mechanisms besides transduction and transformation, such as conjugation, are also significant for gene transfer (Paul, 1999). However, it becomes more appreciated that not only bacterial but also viral genes might be involved in transferring morphological and metabolic traits. Indeed, it has been suggested that '. . . viruses rather than bacteria massively explore sequence space' (Villarreal, 2001).

Conclusions

The consequences of gene transfer differ greatly depending on whether it happens within or among species (Torsvik *et al*., 2002). Gene transfer within species should slow down diversification by increasing genetic similarity. This may result in higher standing stocks of (more or less) identical genomes and thus in a higher survival chance depending on the stability of the environment. Gene transfer across species and genus barriers should promote adaptation and increase diversity. For example, acquiring genes may open new ecological niches (Martin, 1999; Ochman *et al*., 2000) and increase the geographical distribution of species. The new niches would also allow for gene transfer with prokaryotic species in these niches.

Although we only know a little about the detailed mechanisms, it seems likely that viruses shape the standing stock and fluxes of genetically encoded information. This should influence the ecology as well as the evolution of species (DeFilippis and Villarreal, 2000). In the theory of genetic mosaicism, dsDNA phages have access to the global phage DNA pool by lateral gene transfer. This access is not uniform, e.g. because of host range barriers, thus representing '…a sort of random walk through phylogenetic space' (Hendrix *et al*., 1999). If it holds true that host ranges can be much broader than assumed (Chiura, 1997; Jensen *et al*., 1998; Wichels *et al*., 1998), this random walk could turn out to be less random and more sprint-like than previously thought. Owing to lateral gene transfer, prokaryotes might also be seen as a 'global superorganism' (Doolittle, 1999). On account of a viral linkage to the tree of life (Villarreal, 2001), it is maybe more appropriate to regard all microorganisms including viruses as a 'superorganism' or part of a global DNA pool, which can in principle be accessed by all species. Thus, virus-mediated gene transfer and viral genes considerably extend the possibilities of generating genetic variability as a target of selection beyond that caused by point mutations, recombination and sexual exchange. Such a 'Virio-Darwinism' might explain evolutionary changes and inventions, which would be hard to explain otherwise. Indeed, viruses seem to be involved in major evolutionary inventions such as the origin of mammals or eukaryotes (Villarreal and DeFilippis, 2000; Villarreal, 2001).

Studies on genetic diversity and functional aspects of microorganisms are still sparse (Lee *et al*., 1999; Ouverney and Fuhrman, 1999; Cottrell and Kirchman, 2000), and no studies are available linking gene flux to ecological and biogeochemical processes in aquatic systems. Overall, the links between studies on information (e.g. genetic diversity, gene flux) and energy and matter transfer (e.g. carbon and nutrient cycling) are still rather spurious in the fields of aquatic microbial ecology and biogeochemistry. We suggest that (i) including the information aspect of life will greatly enhance our understanding of diversification and microbial food web interactions and (ii) viral genes, virus-mediated gene transfer and viral infection play a crucial role in these processes.

Acknowledgements

An ATIPE grant from the French Science Organization (CNRS) and a European Commission grant (BASICS, EVK3-CT-2002-00078) to M.G.W. supported this work. M.G.W. is grateful to the participants of the CIESM workshop on 'The Ecology of Marine Viruses' for stimulating discussions. Numerous colleagues contributed in shaping the ideas presented; however, they are not responsible for misinterpretations. The comments of three anonymous reviewers improved the manuscript.

References

Ackermann, H.-W. (1999) Tailed bacteriophages: the order *Caudovirales*. *Adv Virus Res* **51:** 135–201.
Ackermann, H.-W., and DuBow, M.S. (1987) *Viruses of Prokaryotes*, Vol. I**.** General Properties of Bacteriophages. Boca Raton: CRC Press.
Arrieta, J.M., and Herndl, G.J. (2002) Changes in bacterial β-glucosidase diversity during a coastal phytoplankton bloom. *Limnol Oceanogr* **47:** 594–599.
Azam, F. (1998) Microbial control of oceanic carbon flux: the plot thickens. *Science* **280:** 694–696.
Bergh, Ø., Børsheim, K.Y., Bratbak, G., and Heldal, M. (1989) High abundance of viruses found in aquatic environments. *Nature* **340:** 467–468.
Breitbart, M., Salamon, P., Andresen, B., Mahaffy, J.M., Segall, A.M., Mead, D., *et al.* (2002) Genomic analysis of uncultured marine viral communities. *Proc Natl Acad Sci USA* **99:** 14250–14255.
Brüssow, H., and Hendrix, R.W. (2002) Phage genomics: small is beautiful. *Cell* **108:** 13–16.
Button, D.K., Robertson, B.R., Lepp, P.W., and Schmidt, T.M. (1998) A small, dilute-cytoplasm, high-affinity, novel bacterium isolated by extinction culture and having kinetic constants compatible with growth at ambient concentrations of dissolved nutrients in seawater. *Appl Environ Microbiol* **64:** 4467–4476.
Chen, F., and Lu, J. (2002) Genomic sequence and evolution of marine cyanophage P60: a new insight on lytic and lysogenic phage. *Appl Environ Microbiol* **68:** 2589–2594.

Chen, F., and Suttle, C.A. (1995) Amplification of DNA polymerase gene fragments from viruses infecting microalgae. *Appl Environ Microbiol* **61:** 1274–1278.

Chiura, H.X. (1997) Generalized gene transfer by virus-like particles from marine bacteria. *Aquat Microb Ecol* **13:** 75–83.

Chiura, H., Velimirov, B., and Kogure, K. (2000) Virus-like particles in microbial population control and horizontal gene transfer in the aquatic environment. In *Microbial Biosystems*. Bell, C., Brylinsky, M., and Johnson-Green, P. (eds). Halifax: Atlantic Canada Society for Microbial Ecology, pp. 145–151.

Clarke, K. (1998) Virus particle production in lysogenic bacteria exposed to protozoan grazing. *FEMS Microbiol Lett* **166:** 1777–1180.

Cottrell, M., and Kirchman, D. (2000) Natural assemblages of marine proteobacteria and members of the *Cytophaga–Flavobacter* cluster consuming low- and high-molecular-weight dissolved organic matter. *Appl Environ Microbiol* **66:** 1692–1697.

Curtis, T.P., Sloan, W.T., and Scannell, J.W. (2002) Estimating prokaryotic diversity and its limits. *Proc Natl Acad Sci USA* **99:** 10494–10499.

Dahllof, I., Baillie, H., and Kjelleberg, S. (2000) rpoB-based microbial community analysis avoids limitations inherent in 16S rRNA gene intraspecies heterogeneity. *Appl Environ Microbiol* **66:** 3376–3380.

DeFilippis, V., and Villarreal, L. (2000) An introduction to the evolutionary ecology of viruses. In *Viral Ecology*. Hurst, C. (ed.). San Diego: Academic Press, pp. 125–208.

Doolittle, W.F. (1999) Phylogenetic classification and the universal tree. *Science* **284:** 2124–2129.

Edlin, G., Lin, L., and Kudra, R. (1975) λ lysogens of *E. coli* reproduce more rapidly than non-lysogens. *Nature* **255:** 735–737.

Fuhrman, J.A. (1999) Marine viruses and their biogeochemical and ecological effects. *Nature* **399:** 541–548.

Fuhrman, J., and Noble, R. (2000) Causative agents of bacterial mortality and the consequences to marine food webs. In *Microbial Biosystems*. Bell, C., Brylinsky, M., and Johnson-Green, P. (eds). Halifax: Atlantic Canada Society for Microbial Ecology, pp. 145–151.

Fuhrman, J.A., and Suttle, C.A. (1993) Viruses in marine planktonic systems. *Oceanography* **6:** 51–63.

Fuller, N.J., Wilson, W.H., Joint, I.R., and Mann, N.H. (1998) Occurrence of a sequence in marine cyanophages similar to that of T4 g20 and its application to PCR-based detection and quantification techniques. *Appl Environ Microbiol* **64:** 2051–2060.

Furuta, M., Schrader, J.O., Schrader, H.S., Kokjohn, T.A., Nyaga, S., McCullough, A.K., *et al.* (1997) Chlorella virus PBCV-1 encodes a homolog of the bacteriophage T4 UV damage repair gene denV. *Appl Environ Microbiol* **63:** 1551–1556.

Giovannoni, S., and Rappé, M. (2000) Evolution, diversity, and molecular ecology of marine prokaryotes. In *Microbial Ecology of the Oceans*. Kirchman, D. (ed.). New York: Wiley-Liss, pp. 47–84.

Glöckner, F.O., Zaichikov, E., Belkova, N., Denissova, L., Pernthaler, J., Pernthaler, A., and Amann, R. (2000) Comparative 16S rRNA analysis of lake bacterioplankton reveals globally distributed phylogenetic clusters including an abundant group of actinobacteria. *Appl Environ Microbiol* **66:** 5053–5065.

González, J.M., and Suttle, C.A. (1993) Grazing by marine nanoflagellates on viruses and viral-sized particles: ingestion and digestion. *Mar Ecol Prog Series* **94:** 1–10.

Hagström, A., Pommier, T., Rohwer, F., Simu, K., Stolte, W., Svensson, D., and Zweifel, U.L. (2002) Use of 16S ribosomal DNA for delineation of marine bacterioplankton species. *Appl Environ Microbiol* **68:** 3628–3633.

Hahn, M.W., Lunsdorf, H., Wu, Q., Schauer, M., Hofle, M.G., Boenigk, J., and Stadler, P. (2003) Isolation of novel ultramicrobacteria classified as Actinobacteria from five freshwater habitats in Europe and Asia. *Appl Environ Microbiol* **69:** 1442–1451.

Hayashi, T., Makino, K., Ohnishi, M., Kurokawa, K., Ishii, K., Yokoyama, K., *et al.* (2001) Complete genome sequence of enterohemorrhagic *Escherichia coli* O157:H7 and genomic comparison with a laboratory strain K-12. *DNA Res* **8:** 11–22.

Hedges, J.I. (1992) Global biogeochemical cycles: progress and problems. *Mar Chem* **39:** 67–93.

Hendrix, R. (1999) The long evolutionary reach of viruses. *Curr Biol* **9:** 9914–9917.

Hendrix, R., Smith, M., Burns, R., Ford, M., and Hatfull, G. (1999) Evolutionary relationships among diverse bacteriophages and prophages: all the world's a phage. *Proc Natl Acad Sci USA* **96:** 2192–2197.

Hendrix, R.W., Lawrence, J.G., Hatfull, G.F., and Casjens, S. (2000) The origins and ongoing evolution of viruses. *Trends Microbiol* **8:** 504–508.

Hennes, K.P., Suttle, C.A., and Chan, A.M. (1995) Fluorescently labeled virus probes show that natural virus populations can control the structure of marine microbial communities. *Appl Environ Microbiol* **61:** 3623–3627.

Hughes, J.B., Hellmann, J.J., Ricketts, T.H., and Bohannan, B.J. (2001) Counting the uncountable: statistical approaches to estimating microbial diversity. *Appl Environ Microbiol* **67:** 4399–4406.

Jensen, E., Schrader, H., Rieland, B., Thompson, T., Lee, K., Nickerson, K., and Kokjohn, T. (1998) Prevalence of broad-host-range lytic bacteriophages of *Spherotilus natans*, *Escherichia coli*, and *Pseudomonas aeruginosa*. *Appl Environ Microbiol* **64:** 575–580.

Jiang, S.C., and Paul, J.H. (1996) Occurrence of lysogenic bacteria in marine microbial communities as determined by prophage induction. *Mar Ecol Prog Series* **142:** 27–38.

Jiang, S.C., and Paul, J.H. (1998) Gene transfer by transduction in the marine environment. *Appl Environ Microbiol* **64:** 2780–2787.

Karner, M.B., DeLong, E.F., and Karl, D.M. (2001) Archaeal dominance in the mesopelagic zone of the Pacific Ocean. *Nature* **409:** 507–510.

Larsen, A., Castberg, T., Sandaa, R., Brussaard, C., Egge, J., Heldal, M., *et al.* (2001) Population dynamics and diversity of phytoplankton, bacteria and viruses in a seawater enclosure. *Mar Ecol Prog Series* **221:** 47–57.

Lawrence, J.G., and Ochman, H. (1998) Molecular archaeology of the *Escherichia coli* genome. *Proc Natl Acad Sci USA* **95:** 9413–9417.

Lawrence, J.G., Hatfull, G.F., and Hendrix, R.W. (2002)

Imbroglios of viral taxonomy: genetic exchange and failings of phenetic approaches. *J Bacteriol* **184:** 4891–4905.

Lebaron, P., Servais, P., Troussellier, M., Courties, C., Vives-Rego, J., Muyzer, G., *et al.* (1999) Changes in bacterial community structure in seawater mesocosms differing in their nutrient status. *Aquat Microb Ecol* **19:** 255–267.

Lee, N., Nielsen, P.H., Andreasen, K.H., Juretschko, S., Nielsen, J.L., Schleifer, K.H., and Wagner, M. (1999) Combination of fluorescent *in situ* hybridization and microautoradiography – a new tool for structure–function analyses in microbial ecology. *Appl Environ Microbiol* **65:** 1289–1297.

Lin, L., Bitner, R., and Edlin, G. (1977) Increased reproductive fitness of *Escherichia coli* lambda lysogens. *J Virol* **21:** 554–559.

Long, R., and Azam, F. (2001) Microscale patchiness of bacterioplankton assemblage richness in seawater. *Aquat Microb Ecol* **26:** 101–113.

Margalef, R. (1997) *Our Biosphere*. Oldendorf/Luhe: Inter-Research.

Martin, W. (1999) Mosaic bacterial chromosomes: a challenge en route to a tree of genomes. *Bioessays* **21:** 99–104.

Middelboe, M. (2000) Bacterial growth rate and marine virus-host dynamics. *Microb Ecol* **40:** 114–124.

Middelboe, M., Hagström, A., Blackburn, N., Sinn, B., Fischer, U., Borch, N., *et al.* (2001) Effects of bacteriophages on the population dynamics of four strains of pelagic marine bacteria. *Microb Ecol* **42:** 395–406.

Moineau, S., Pandian, S., and Klaenhammer, T. (1994) Evolution of a lytic bacteriophage via DNA acquisition from the *Lactococcus lactis* chromosome. *Appl Environ Microbiol* **60:** 1832–1841.

Moineau, S., Pandian, S., and Klaenhammer, T.R. (1995) Specific chromosomal sequences can contribute to the appearance of a new lytic bacteriophage in *Lactococcus*. *Dev Biol Stand* **85:** 577–580.

Morris, R.M., Rappé, M.S., Connon, S.A., Vergin, K.L., Siebold, W.A., Carlson, C.A., and Giovannoni, S.J. (2002) SAR11 clade dominates ocean surface bacterioplankton communities. *Nature* **420:** 806–810.

Murray, A.E., Hollibaugh, J.T., and Orrego, C. (1996) Phylogenetic compositions of bacterioplankton from two California estuaries by denaturing gradient gel electrophoresis of 16R rDNA fragments. *Appl Environ Microbiol* **62:** 2676–2680.

Ochman, H., Lawrence, J., and Groisman, E. (2000) Lateral gene transfer and the nature of bacterial innovation. *Nature* **405:** 299–304.

Ohnishi, M., Kuokawa, K., and Hayashi, T. (2001) Diversification of *Escherichia coli* genomes: are bacteriophages the main contributors? *Trends Microbiol* **9:** 481–485.

Ouverney, C.C., and Fuhrman, J.A. (1999) Combined microautoradiography-16S rRNA probe technique for determination of radioisotope uptake by specific microbial cell types *in situ*. *Appl Environ Microbiol* **65:** 1746–1752.

Øvreås, L., Bournde, D., Sandaa, R.-A., Casamayor, E., Benlloch, S., Goddard, V., *et al.* (2003) Response of bacterial and viral communities to nutrient manipulations in seawater mesocosms. *Aquat Microb Ecol* **31:** 109–121.

Paul, J.H. (1999) Microbial gene transfer. *J Mol Microbiol Biotechnol* **1:** 45–50.

Paul, J.H., Sullivan, M.B., Segall, A.M., and Rohwer, F. (2002) Marine phage genomics. *Comp Biochem Physiol B Biochem Mol Biol* **133:** 463–476.

Peduzzi, P., and Weinbauer, M.G. (1993a) The submicron size fraction of seawater containing high numbers of virus particles as bioactive agent in unicellular plankton community successions. *J Plankton Res* **15:** 1375–1386.

Peduzzi, P., and Weinbauer, M.G. (1993b) Effect of concentrating the virus-rich 2–200 nm size fraction of seawater on the formation of algal flocs (marine snow). *Limnol Oceanogr* **38:** 1562–1565.

Pennisi, E. (1998) Genome data shake tree of life. *Science* **280:** 672–674.

Pinhassi, J., Azam, F., Hemphälä, J., Long, R.A., Martinez, J., Zweifel, U.L., and Hagström, Å. (1999) Coupling between bacterioplankton species composition, population dynamics, and organic matter degradation. *Aquat Microb Ecol* **17:** 13–26.

Proctor, L.M., and Fuhrman, J.A. (1990) Viral mortality of marine bacteria and cyanobacteria. *Nature* **343:** 60–62.

Proctor, L.M., and Fuhrman, J.A. (1991) Roles of viral infection in organic particle flux. *Mar Ecol Prog Series* **69:** 133–142.

Rappé, M.S., Connon, S.A., Vergin, K.L., and Giovannoni, S.J. (2002) Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. *Nature* **418:** 630–633.

Regenmortel, M.H.V.V. (1992) Concept of virus species. *Biodiversity Conserv* **1:** 263–266.

Riemann, L., Steward, G.F., and Azam, F. (2000) Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. *Appl Environ Microbiol* **66:** 578–587.

Ripp, S., and Miller, R.V. (1995) Effects of suspended particles on the frequency of transduction among *Pseudomonas aeruginosa*. a freshwater environment. *Appl Environ Microbiol* **61:** 1214–1219.

Rohwer, F., and Edwards, R. (2002) The phage proteomic tree: a genome-based taxonomy for phage. *J Bacteriol* **184:** 4529–4535.

Rohwer, F., Segall, A., Steward, G., Seguritan, V., Breitbart, M., Wolven, F., and Azam, F. (2000) The complete genomic sequence of marine phage Roseophage SIO1 shares homology with nonmarine phages. *Limnol Oceanogr* **45:** 408–418.

Rossello-Mora, R., and Amann, R. (2001) The species concept for prokaryotes. *FEMS Microbiol Rev* **25:** 39–67.

Saye, D.J., Ogunseitan, O., Sayler, G.S., and Miller, R.V. (1987) Potential for transduction of plasmids in a natural freshwater environment: effect of plasmid donor concentration and natural microbial community on transduction in *Pseudomonas aeruginosa*. *Appl Environ Microbiol* **53:** 9897–9995.

Short, S., and Suttle, C. (2002) Sequence analysis of marine virus communities reveals that groups of related algal viruses are widely distributed in nature. *Appl Environ Microbiol* **68:** 1290–1296.

Simon, M., Grossart, H.-P., Schweitzer, B., and Ploug, H. (2002) Microbial ecology of organic aggregates in aquatic ecosystems. *Aquat Microb Ecol* **28:** 175–211.

Stackebrandt, E., and Goebel, B.M. (1994) Taxonomic note: a place for DNA–DNA reassociation and 16S rRNA

sequence analysis in the present species definition in bacteriology. *Appl Environ Microbiol* **44:** 846–849.

Staley, J.T., and Konopka, A. (1985) Measurement of *in situ* activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. *Annu Rev Microbiol* **39:** 321–346.

Steward, C., and Azam, F. (2000) Analysis of marine viral assemblages. In *Microbial Biosystems*. Bell, C., Brylinsky, M., and Johnson-Green, P. (eds). Halifax: Atlantic Canada Society for Microbial Ecology, pp. 159–165.

Steward, G., Montiel, J., and Azam, F. (2000) Genome size distributions indicate variability and similarity among marine viral assemblages from diverse environments. *Limnol Oceanogr* **45:** 1697–1706.

Suttle, C.A. (1992) Inhibition of photosynthesis in phytoplankton by the submicron size fraction concentrated from seawater. *Mar Ecol Prog Series* **87:** 105–112.

Suttle, C. (2000a) Ecological, evolutionary, and geochemical consequences of viral infection of cyanobacteria and eukaryotic algae. In *Viral Ecology*. Hurst, C. (ed.). San Diego: Academic Press, pp. 247–296.

Suttle, C. (2000b) Cyanophages and their role in the ecology of cyanobacteria. In *The Ecology of Cyanobacteria*. Whitton, B., and Potts, M. (eds). The Netherlands: Kluwer Academic Publishers, pp. 563–589.

Suttle, C.A., and Chan, A.M. (1994) Dynamics and distribution of cyanophages and their effect on marine *Synechococcus* spp. *Appl Environ Microbiol* **60:** 3167–3174.

Suttle, C.A., Chan, A.M., and Cottrell, M.T. (1990) Infection of phytoplankton by viruses and reduction of primary productivity. *Nature* **347:** 467–469.

Thingstad, T.F., and Lignell, R. (1997a) Theoretical models for the control of bacterial growth rate, abundance, diversity and carbon demand. *Aquat Microb Ecol* **13:** 19–27.

Thingstad, T., and Lignell, R. (1997b) Trophic interactions controlling the diversity in pelagic microbial food webs. In *Progress in Microbial Ecology*. Martins, M. (ed.). São Paulo: Brazilian Society for Microbiology, pp. 107–114.

Thingstad, T.F., Heldal, M., Bratbak, G., and Dundas, I. (1993) Are viruses important partners in pelagic food webs? *Trends Ecol Evol* **8:** 209–213.

Torsvik, V., Ovreas, L., and Thingstad, T.F. (2002) Prokaryotic diversity-magnitude, dynamics, and controlling factors. *Science* **296:** 1064–1066.

Van Hannen, E.J., Zwart, G., van Agterveld, M.P., Gons, H.J., Ebert, J., and Laanbroek, H.J. (1999) Changes in bacterial and eukaryotic community structure after mass lysis of filamentous cyanobacteria associated with viruses. *Appl Environ Microbiol* **65:** 795–801.

Villarreal, L. (2001) Persisting viruses could play role in driving host evolution. *Am Soc Microbiol News* **67:** 501–507.

Villarreal, L.P., and DeFilippis, V.R. (2000) A hypothesis for DNA viruses as the origin of eukaryotic replication proteins. *J Virol* **74:** 7079–7084.

Waldor, M., and Mekalanos, J. (1996) Lysogenic conversion by a filamentous phage encoding cholera toxin. *Science* **272:** 1910–1914.

Ward, D. (1998) A natural species concept for prokaryotes. *Curr Opin Microbiol* **1:** 271–277.

Waterbury, J.B., and Valois, F.W. (1993) Resistance to co-occurring phages enables marine Synechococcus communities to coexist with cyanophages abundant in seawater. *Appl Environ Microbiol* **59:** 3393–3399.

Weinbauer, M. (2003) Ecology of prokaryotic viruses. *FEMS Microbiol Ecol* (in press).

Weinbauer, M.G., and Höfle, M.G. (1998) Distribution and life strategies of two bacterial populations in a eutrophic lake. *Appl Environ Microbiol* **64:** 3776–3783.

Weinbauer, M.G., and Suttle, C.A. (1996) Potential significance of lysogeny to bacteriophage production and bacterial mortality in coastal waters of the Gulf of Mexico. *Appl Environ Microbiol* **62:** 4374–4380.

Weinbauer, M., Brettar, I., and Höfle, M. (2003) Lysogeny and virus-induced mortality of bacterioplankton in surface, deep, and anoxic waters. *Limnol Oceanogr* **48:** 1457–1465.

Wheelis, M.L., Kandler, O., and Woese, C.R. (1992) On the nature of global classification. *Proc Natl Acad Sci USA* **89:** 2930–2934.

Wichels, A., Biel, S.S., Gelderblom, H.R., Brinkhoff, T., Muyzer, G., and Schütt, C. (1998) Bacteriophage diversity in the North Sea. *Appl Environ Microbiol* **64:** 4128–4133.

Wilhelm, S.W., and Suttle, C.A. (1999) Viruses and nutrient cycles in the sea. *Bioscience* **49:** 781–788.

Wilson, E. (1992) *The Diversity of Life*. New York: W.W. Norton & Co.

Wilson, W., Fuller, N., Joint, I., and Mann, N. (1999) Analysis of cyanophage diversity and population structure in a south-north transect of the Atlantic ocean. *Bull Inst Océanogr Monaco* **19:** 209–216.

Wintzingerode, F., Göbel, U.B., and Stackebrandt, E. (1997) Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. *FEMS Microbiol Rev* **21:** 213–229.

Woese, C.R. (1987) Bacterial evolution. *Microbiol Rev* **51:** 221–271.

Wommack, K.E., and Colwell, R.R. (2000) Virioplankton: viruses in aquatic ecosystems. *Microbiol Mol Biol Rev* **64:** 69–114.

Wommack, K.E., Ravel, J., Hill, R.T., and Colwell, R.R. (1999a) Hybridization analysis of Chesapeake Bay virioplankton. *Appl Environ Microbiol* **65:** 241–250.

Wommack, K.E., Ravel, J., Holl, R.T., Chun, J., and Colwell, R.R. (1999b) Population dynamics of Chesapeake Bay virioplankton: total-community analysis by pulse-field gel electrophoresis. *Appl Environ Microbiol* **65:** 231–240.

Zhong, Y., Chen, F., Wilhelm, S., Poorvin, L., and Hodson, R. (2002) Phylogenetic diversity of marine cyanophage isolates and natural virus communities as revealed by sequences of viral capsid assembly protein gene g20. *Appl Environ Microbiol* **68:** 1576–1584.